Title: Cloning Gate+LuxI+GFP on pSB1C3
Goal: Create a gated reporter
Date range: August-September 2015
Members that worked on project: Michael Donovan

Results: Successfully cloned part

New part number: BBa_K1718005
Part size: 1851

PCR of BBa_K1718002:

- Purpose: Isolate the Gate piece from its backbone
- PCR polymerase: Q5 HF 2x Master Mix.
- Primers used: VF2 and VR sequencing primers.

Reagent	**25 ul reaction**	**Concentration**
2x Q5	12.25 ul	1 x
10 uM F. Primer	1.25 ul	0.5 uM
10 uM R. Primer	1.25 ul	0.5 uM
DNA (140 ng/ul)	2 ul	0.249 ng
Q5 polymerase	.25 ul	0.02 U/ul
Water	8.0 ul	

Reaction conditions:

Step	**Temp (C)**	**Time**
I. Denature	98	30 s.
35 cycles	98	10 s
	65	15 s
	72	50 s

Final extension	72	2 min.

- Annealing temp: 65°C
- Extension time: 50 s

PCR clean-up:

- Elution volume: 30ul
- Concentration: 80.0 ng/ul

Digestion:

- For Ligation
- Digestion of: PCR product of BBa_K1718002 on 1C3 (give biobrick number if possible, otherwise describe)

Reagent	50 ul reaction	Concentration
Cutsmart Buffer	5 ul	1 x
EcoRI-HF	1 ul	10 units
SpeI	1 ul	10 units
DNA (80ng/ul)	6 ul	480 ng
DPN1	1 ul	10 units
Water	36 ul	

- Digestion of: BBa_K1718004

Reagent	50 ul reaction	Concentration
Cutsmart Buffer	5 ul	1 x
EcoRI-HF	1 ul	10 units
XbaI	1 ul	10 units
DNA (260ng/ul)	2 ul	520 ng
Water	41 ul	

Ligation:

- BBa_K1718002 with E and S, BBa_K1718004 on 1C3 with E and X
- 2 ul BBa_K1718004, 10 ul BBa_K1718002

Component	20 ul reaction	Concentration/amount
10XT4 DNA Ligase Buffer	2 ul	1 x
Vector DNA	2 ul	
Insert DNA	10 ul	
T4 DNA Ligase	1 ul	
Nuclease-free water	5 ul	

Let reaction run for 14 hours at 16 C then heat shock at 65 C for 10 minutes.
Run a negative insert control.

Transformation:

- 10 ul of ligation product from into 40ul of 5 alpha competent cells.
- Let cells sit with DNA on ice for 30 minutes.
- Heat shock at 42°C for 45 seconds.
- Put on ice for 5 minutes.
- Treat cells with 950ul of SOC.
- Put in incubator at 37°C for 1 to 2 hours.
- Spread 100ul of cells onto chloramphenicol resistance plates (170ng/ul concentration)
- Spin tube gently for 2 minutes, remove 750 ul of SOC, resuspend pellet and plate 100 ul

Overnight: Choose 2 colonies from each plate
Mini-prep:

- 1: 543 ng/ul 2: 468 ng/ul 3: 520 ng/ul 4: 603 ng/ul

Colonies on the plate fluoresced green, indicating that the gate is leaky.
Gel and sequencing supported the existence of a correct construct.

Sequence:

- Primers used
- Correct sequence

Part submission:

- BBa_K1718005